Urokinase Plasminogen Activator Is Expressed by Basal Keratinocytes Before Interstitial Collagenase, Stromelysin-1, and Laminin-5 in Experimentally Induced Dermatitis Herpetiformis Lesions

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We studied the temporal expression of interstitial collagenase, stromelysin-1 and -2, and urokinase plasminogen activator (uPA) mRNAs by *in situ* hybridization in eight patients with dermatitis herpetiformis. To induce blisters, 50% potassium iodide patch tests were performed, and serial biopsy specimens were taken at 4, 12, and 24 h. Additional samples were taken from occasional spontaneous blisters. Components of the basement membrane, laminin-5, laminin-1, and type VII collagen, were examined immunohistochemically in relation to matrix metalloproteinase expression. At 12 h, when no blisters were seen, uPA mRNA was present in basal keratinocytes in five of eight samples, whereas interstitial collagenase and stromelysin-1 mRNA were not detected. At this time, immunohistochemistry failed to show changes in the basement membrane. At 24 h, uPA, collagenase, and stromelysin-1 mRNAs were present in basal keratinocytes, suggesting an activation of latent forms of the two latter enzymes by the uPA-plasmin pathway. Signal for stromelysin-2 was not detected. Furthermore, disruptions of laminin-1 and type VII collagen were evident. The data suggest that stromelysin-1 and interstitial collagenase may contribute to the degradation of basement membrane in dermatitis herpetiformis. Intracellular staining for laminin-5 co-localized with collagenase mRNA in basal keratinocytes. Because laminin-5 is essential for adhesion of keratinocytes to basement membrane and for establishment of focal adhesions on migrating cells, its production may reflect a regenerative response after the destruction of basement membrane components. *Key words: metalloproteinase/bullous disease. J Invest Dermatol 108:7–11, 1997*

Dermatitis herpetiformis (DH) is a chronic cutaneous disorder characterized by perivascular inflammatory infiltrates and accumulations of neutrophils at the papillary dermis, with subsequent subepidermal blister formation. We have previously reported that the expression of the matrix metalloproteinases interstitial collagenase (MMP-1) and stromelysin-1 (MMP-3) is enhanced in basal keratinocytes of DH lesions (Airola *et al*, 1995). These proteinases are encountered much more frequently in DH blisters than in other bullous disorders such as epidermolysis bullosa, porphyria cutanea tarda, pemphigoid, or pemphigus (Ståhle-Bäckdahl *et al*, 1994; Saarialho-Kere *et al*, 1995). To study in which order these enzymes are expressed during DH blister formation, we produced DH blisters with 50% potassium iodide patch tests (Salo *et al*, 1970; Haffenden *et al*, 1980; Blenkinsopp *et al*, 1983) in 12 DH patients and took serial biopsy specimens at 4, 12, and 24 h.

In vitro studies have shown that plasmin converts procollagenase and prostromelysin-1 to active enzymes (Murphy *et al*, 1992), and this is preceded by the conversion of plasminogen to plasmin by urokinase plasminogen activator (uPA). Thus, we were particularly interested in determining whether the production of uPA precedes that of collagenase and stromelysin-1 during DH blister formation. Because production of stromelysin-2 in wounded epidermis co-localizes with collagenase (Saarialho-Kere *et al*, 1994), its expression was studied also. Furthermore, immunohistochemical staining for laminin-5 (kalinin), laminin-1, and type VII collagen was performed on serial sections to examine the changes occurring in the basement membrane zone during development of blisters and to study the spatial correlation of basement membrane proteins and proteinase mRNAs in lesions of DH.

We report here that in experimentally induced DH lesions, uPA mRNA was produced by basal keratinocytes by 12 h, at which time collagenase and stromelysin-1 were still undetectable. At 24 h, when neutrophilic abscesses and subepidermal blisters had developed, a constant signal for all three proteases was present, resembling the pattern of expression in spontaneous blisters. Simultaneously, the normal bandlike staining for laminin-5 and laminin-1 was disrupted at lesional areas, and shorter discontinuities were evident in the staining pattern of type VII collagen. Intracellular staining for laminin-5 localized in collagenase-positive basal keratinocytes in both experimentally induced and spontaneous blisters.

Manuscript received February 20, 1996; revised June 10, 1996; accepted for publication August 1, 1996.

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Abbreviations: DH, dermatitis herpetiformis; TIMP, tissue inhibitor of metalloproteinase; uPA, urokinase plasminogen activator.

MATERIALS AND METHODS

Tissues Biopsy specimens were obtained from 12 patients whose diagnosis of DH had been confirmed clinically and by immunofluorescence studies showing granular IgA deposits in the papillary dermis. Most patients were on a partial gluten-free diet, and those taking dapsone (10/12) were asked to stop their medication 24 h before the study. The following morning, three aluminum chambers (Finnchamber; Epitest Ltd., Hyrylä, Finland) containing 50% potassium iodide and one petrolatum control chamber were placed on the buttocks of each patient, as described previously (Reitamo *et al*, 1981). Serial biopsy specimens were taken from potassium iodide test areas at 4, 12, and 24 h and also from four spontaneous blisters. Eight patients' specimens were processed as formalin-fixed, paraffin-embedded samples, and four patients' specimens were fresh frozen. Samples taken from 50% potassium iodide test areas in four healthy volunteers were also examined for control purposes.

***In Situ* Hybridization** The production and specificity of the anti-sense human interstitial collagenase, stromelysin-1 and -2, and uPA cRNA probes have been described (Saarialho-Kere *et al*, 1992, 1994; Airola *et al*, 1995). A 518-bp fragment corresponding to positions 382 to 900 from the 5′ end of the human TIMP-3 (tissue inhibitor of metalloproteinase) cDNA (Silbiger *et al*, 1994) was generated by polymerase chain reaction. The fragment was designed with a T7 RNA polymerase recognition element at the 3′ end and an SP6 polymerase recognition element at the 5′ end. Both sense and anti-sense probes were transcribed from this polymerase chain reaction product. As a control for nonspecific hybridization, tissue sections were also hybridized with sense RNA transcribed from a bovine tropoelastin cDNA. The validity of this probe as a negative control has been confirmed by Northern blot (Ståhle-Bäckdahl and Parks, 1993) and *in situ* hybridization assays (Saarialho-Kere *et al*, 1992).

In vitro transcribed anti-sense and sense RNA probes were labeled with [α-^{35}S]UTP (Saarialho-Kere *et al*, 1993b). Sections were hybridized with probes (2.5-3 $\times$ 10^4 cpm/μl of hybridization buffer) and were washed under stringent conditions, including treatment with RNase A, as described (Prosser *et al*, 1989; Saarialho-Kere *et al*, 1993a). After autoradiography for 13-25 d, the photographic emulsion was developed and the slides were stained with hematoxylin and eosin. Samples known to be positive for a certain probe were used as positive controls, and a sense probe was used as a negative control in each experiment. Each sample was hybridized in two or three different experiments to exclude false-negative results. The slides were independently analyzed by two investigators.

Immunohistochemistry Immunostaining for laminin-5 and laminin-1 was performed on sections parallel to those used for *in situ* hybridizations. The peroxidase-antiperoxidase technique was used with diaminobenzidine as chromogenic substrate, as described (Saarialho-Kere *et al*, 1993b; Ståhle-Bäckdahl and Parks, 1993). Anti-human laminin-5 polyclonal antibodies (Pyke *et al*, 1995), monoclonal anti–laminin-1 antibody (Lam-89; L8271, Sigma, St. Louis, MO), and monoclonal anti–type VII collagen antibody (MAB1345; Chemicon, Temecula, CA) were used to stain these basement membrane proteins. Laminin-5 antibody was diluted 1:250 and reacted overnight at 4°C, and laminin-1 was diluted 1:1000 and reacted for 1 h at 37°C. Type VII collagen staining was performed on acetone-fixed frozen sections, and the antibody was diluted 1:100 and reacted for 1 h at 37°C. The tissues were counterstained with hematoxylin. Controls were performed with rabbit pre-immune serum or pre-immune mouse ascites fluid.

RESULTS

Tissues Lesions produced by 50% potassium iodide were clinically and histologically identical to spontaneous DH blisters. At 4 h, excess lymphocytes were seen in the dermis, and by 12 h their number had increased to form perivascular infiltrates **(Fig 1*A–C*)**. Some areas showed vacuolization of basal keratinocytes. At 24 h, typical features included inflammatory cell infiltrates, neutrophilic abscesses with various stages of multilocular blisters, and papillary edema **(Fig 1*D–H*)**. Most spontaneous blisters examined were unilocular. Clinically, potassium iodide test areas showed erythema, papules, and microvesicles. The specimens taken from four healthy volunteers (three samples at 24 h and one at 48 h) showed mild eczema with lymphocytic infiltration in the upper dermis and pericellular edema of the basal keratinocytes, but no blister formation.

Expression of uPA Precedes That of Collagenase and Stromelysin-1 Eight patients' samples were studied for the expression of interstitial collagenase, stromelysin-1, and uPA mRNAs by *in situ* hybridization. At 4 h, all specimens were negative. At 12 h, five of eight samples showed signals for uPA mRNA **(Fig 1*A*)**, but were negative for collagenase and stromelysin-1 **(Fig 1*B*)**. The signal for uPA mRNA was detected in areas adjacent to inflammatory infiltrates, and at this stage the basement membrane was intact, as determined by continuous staining of laminin-5 **(Fig 1*C*)** and laminin-1 (data not shown). At 24 h, all eight samples were positive for collagenase and stromelysin-1, and seven of eight were positive for uPA **(Fig 1*D–F*)**. The four spontaneous blisters were all positive for collagenase and stromelysin-1, and two were positive for uPA **(Fig 2*B–D*)**. All proteases were expressed by the same populations of basal keratinocytes at the areas of infiltrates, especially by cells surrounding neutrophilic abscesses. Signal for collagenase was also detected in some fibroblast- and macrophage-like stromal cells **(Fig 1*E*)**. Although monocytes/macrophages and polymorphonuclear cells are known to produce uPA (Vassalli *et al*, 1991), mRNA expression in DH was confined to keratinocytes. All specimens were negative for stromelysin-2 mRNA. Seven of the samples were also studied for expression of TIMP-3 mRNA, and no signal was detected.

Negative controls hybridized with sense RNA probe had only background signal **(Fig 1*H*)**. As reported earlier, there is no expression of collagenase, stromelysin-1, or uPA in normal-looking skin of DH patients (Airola *et al*, 1995). No signal for these mRNAs was detected in the four samples of the healthy volunteers (data not shown).

Demonstration of Laminin-5, Laminin-1, and Type VII Collagen by Immunohistochemical Studies Sections adjacent to those used for *in situ* hybridization were stained immunohistochemically for laminin-5 and laminin-1. Laminin-5 localized to the basement membrane zone in samples taken at 4 and 12 h **(Fig 1*C*)**. At 24 h, the linear bandlike staining was disrupted in lesional areas, and intracellular staining was detected in basal keratinocytes **(Figs 1*G*, 2*A*, 3*A*)**. Staining was intense, particularly in the cells surrounding neutrophilic abscesses and in the vicinity of inflammatory infiltrates. Stained cells were more widely distributed than the ones expressing mRNA for collagenase, stromelysin-1, or uPA **(Fig 2*A–D*)**, but represented the same cell populations. Laminin-5 immunostaining and collagenase mRNA co-localized **(Figs 1*E,G*; 2*A,B*)** in individual cells. Samples of healing cutaneous wounds were used as positive controls for laminin-5 (Larjava *et al*, 1993).

Staining for laminin-1 was intact at 12 h, but at 24 h all samples showed various stages of destruction of the basement membrane **(Fig 3*B*)**. Type VII collagen showed more variable results, although it was always localized beneath the blisters **(Fig 3*C*)**. Staining was clearly disrupted in two induced blisters **(Fig 3*C*)**, but was nearly intact in one induced and two spontaneous blisters.

DISCUSSION

Topically applied potassium iodide has been used to induce lesions on the skin of patients with DH for research and diagnostic purposes (Salo *et al*, 1970; Haffenden *et al*, 1980; Blenkinsopp *et al*, 1983). Patients with active disease who are not treated with a gluten-free diet or dapsone are most likely to produce blisters, and the histology of the lesions is similar to that of spontaneous blisters (Blenkinsopp *et al*, 1983). In our study, 11 of the 12 patients showed multiple subepidermal blisters or neutrophilic microabscesses after 24-h application of 50% potassium iodide. The remaining patient did not show these changes but had histology typical of a developing lesion, with inflammatory infiltrates and vacuolization of basal cells. The mechanism by which potassium iodide provokes blistering in DH is not known, but the reaction is characteristic of DH patients, and healthy controls do not develop blisters. In previous studies on other bullous diseases, two patients with bullous pemphigoid and two patients with linear IgA disease were reported to have a negative potassium iodide patch test (Salo *et al*, 1970; Haffenden *et al*, 1980).

In our previous study of archival spontaneous DH blisters, we found that collagenase was expressed constantly by basal keratino-

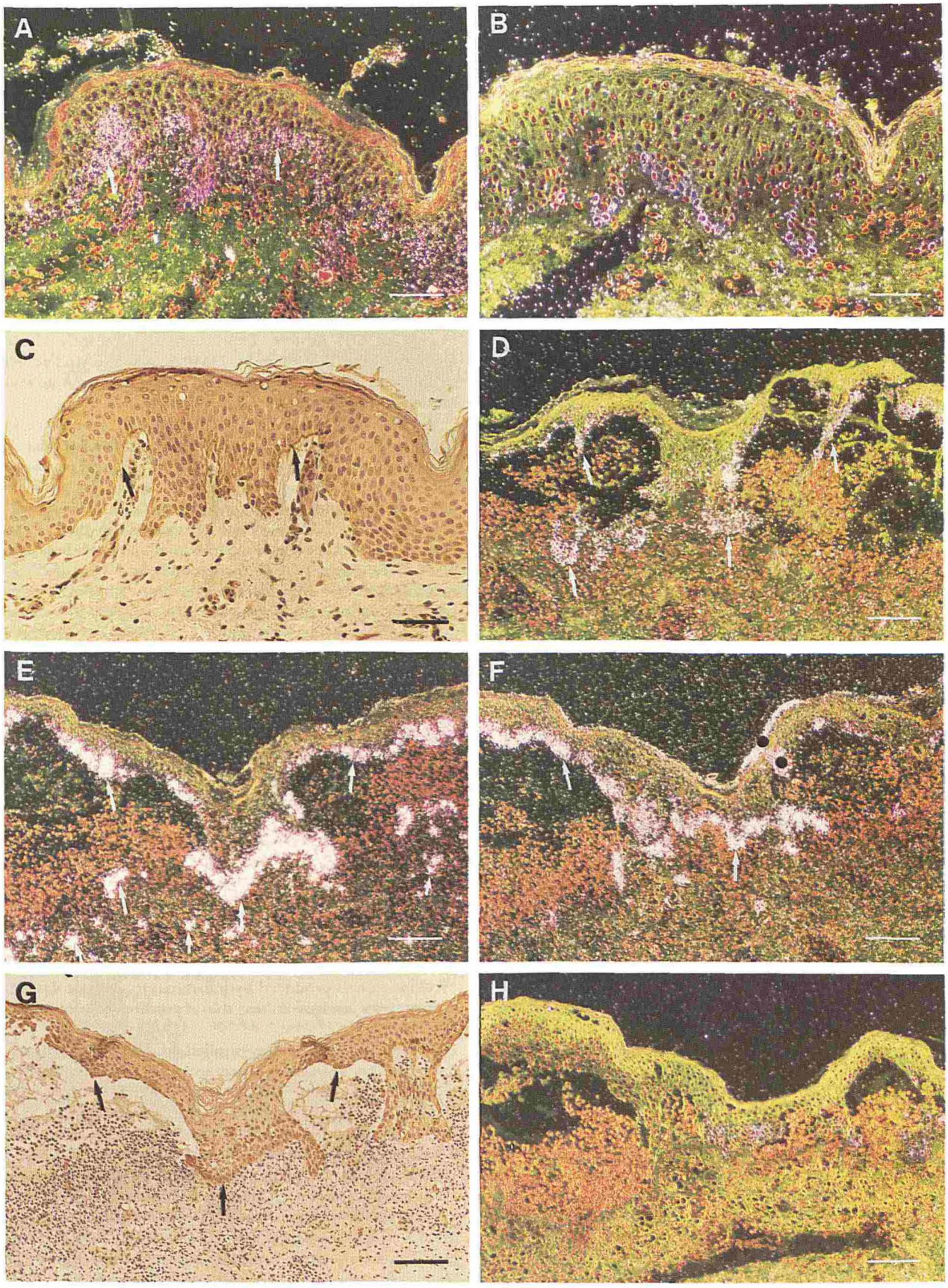

Figure 1. uPA mRNA is produced at 12 h, whereas collagenase and stromelysin-1 appear by 24 h in DH lesions. Blisters were induced with 50% potassium iodide patch tests in DH patients, and biopsy specimens were taken at 4, 12, and 24 h. Serial sections were hybridized with uPA *(A,D)*, stromelysin-1 *(B,F)*, or collagenase anti-sense *(E)* and sense *(H)* probes, or were processed for immunohistochemistry with laminin-5 *(C,G)* antibodies, as described in *Materials and Methods*. *A*, expression of uPA mRNA in basal and suprabasal keratinocytes *(arrows)* at 12 h. Collagenase and stromelysin-1 *(B)* are still undetectable. *C*, immunostaining for laminin-5 reveals a bandlike pattern in the basement membrane *(arrows)*. At 24 h, a consistent signal for uPA *(D)*, collagenase *(E)*, and stromelysin-1 *(F)* mRNAs is present in basal keratinocytes *(arrows)* of samples displaying subepidermal blisters together with neutrophilic infiltrates. *E*, *small arrows* depict signal for collagenase in fibroblast- and macrophage-like stromal cells. *F*, the *dots* mark an autoradiographic artifact. *G*, intracellular production of laminin-5 in basal keratinocytes *(arrows)* co-localizing with collagenase mRNA *(E)*. *H*, a section hybridized with a sense bovine tropoelastin probe is negative. *A,B,D–F,H*, dark-field images; time of autoradiography was 13–25 d. *Scale bars*: *A–C*, 19 μm; *D–H*, 38 μm.

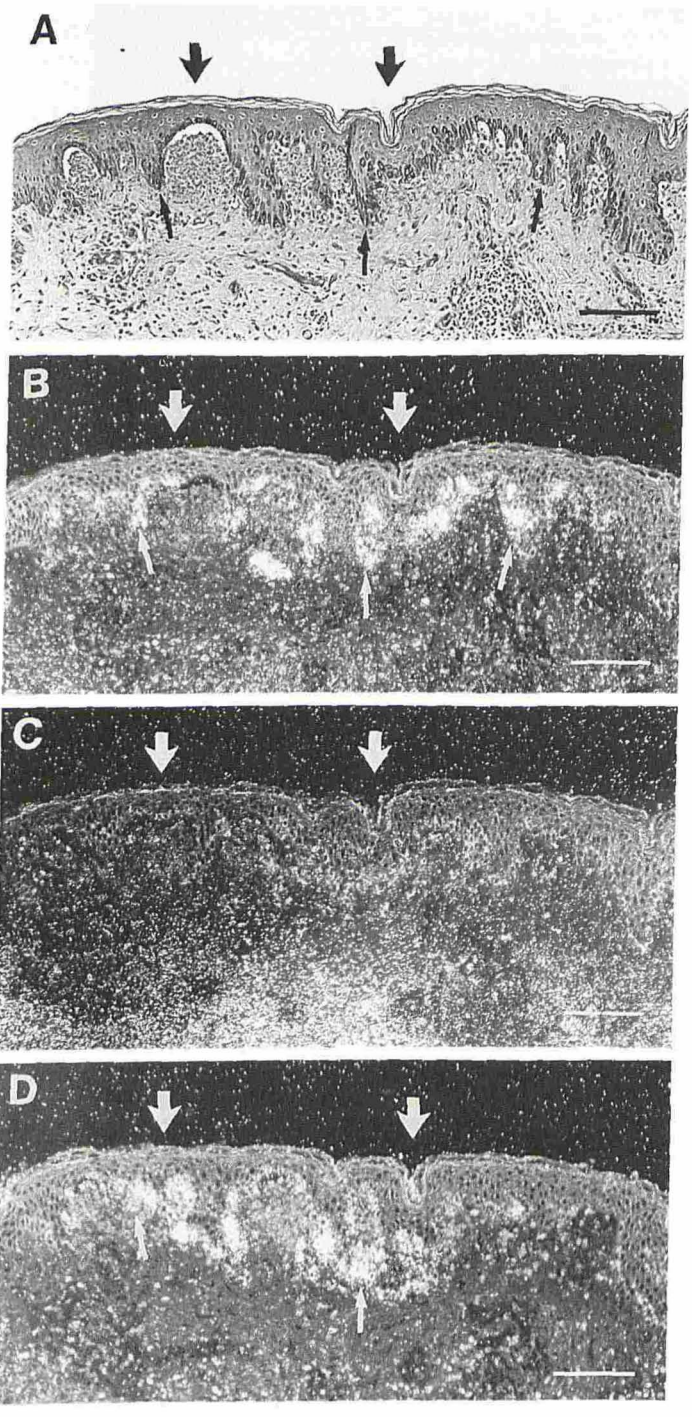

Figure 2. Laminin-5 *immunostaining* co-localizes with collagenase and stromelysin-1 mRNA. *Serial sections were processed for immunohistochemistry with laminin-5 antibodies (A) or hybridized with collagenase (B), uPA (C), or stromelysin-1 (D) anti-sense probes. A, a spontaneous DH lesion showing intracellular staining for laminin-5 (small arrows) in basal keratinocytes in lesional areas. Laminin-5 co-localizes with collagenase (B) and stromelysin-1 (D) mRNA (small arrows), whereas uPA (C) mRNA is not associated with laminin-5 production. Thick arrows mark the corresponding spots. B–D, dark-field images; autoradiographic exposure was for 13–25 d. Scale bars: A–D, 38 μm.*

cytes surrounding neutrophilic abscesses. Expression of stromelysin-1 and uPA was also present in most samples, whereas no signal for matrilysin, 92-kDa gelatinase, or TIMP-1 mRNA was detected. A majority (seven of 11) of the samples were older unilocular blisters with signs of re-epithelialization, whereas in this study, the 24-h samples represented very recent blisters. Thus, it seems that expression of all three enzymes is an early phenomenon in DH and not a secondary event associated with evident epidermal regeneration, as in various other bullous diseases (Saarialho-Kere *et al*, 1995). The mRNA for uPA was induced before any evident changes in the epidermis or basement membrane zone had occurred **(Fig 1*A*)**, at a time when the only histologic findings were inflammatory infiltrates and occasional vacuolization of basal keratinocytes. By 24 h, separation of epidermis and dermis had occurred, and all samples showed expression of collagenase and stromelysin-1. At that point, one experimental and two spontaneous blisters did not contain uPA mRNA, which may reflect time-related changes in expression during the development and healing of DH blisters. Expression of a recently characterized member of the TIMP family, TIMP-3, was also studied because of its ability to inhibit collagenase and stromelysin-1 (Apte *et al*, 1995), but it was not detected.

Stromelysin-1 is capable of degrading both type IV collagen and laminin-1, which are disrupted or absent in lesional DH skin (Karttunen *et al*, 1984; Airola *et al*, 1995), whereas plasmin degrades only laminin-1 among the basement membrane proteins studied here. At 12 h, laminin-1 was still intact in areas with signal for uPA, whereas at 24 h, expression of stromelysin-1 was prominent, particularly in areas where separation of epidermis and dermis took place. This suggests a role for stromelysin-1 in degrading components of the basement membrane, leading to blister formation. The signal for collagenase at 24 h suggests that only keratinocytes that are in contact with a structurally incomplete basement membrane express collagenase **(Figs 1*E,G*; 2*A,B*)**, as shown earlier in suction blisters (Saarialho-Kere *et al*, 1995) and dermal wounds (Saarialho-Kere *et al*, 1993b; Inoue *et al*, 1995).

If one excludes cutaneous wounds (Saarialho-Kere *et al*, 1994), data on stromelysin-2 production in skin diseases is scarce. In DH, stromelysin-2 is not expressed, indicating a different pattern of induction for stromelysin-1 and -2. Indeed, stromelysin-1 together with collagenase and uPA can be induced by cytokines and growth factors, in contrast to stromelysin-2, which is not induced by these agents (Buttice and Kurkinen, 1993). In lesions of DH, increased expression of many such agents has been described, including interleukin-8, endothelial leukocyte adhesion molecules, granulocyte-macrophage colony stimulating factor (Graeber *et al*, 1993), and vascular permeability factor (Brown *et al*, 1995). Furthermore, genetic studies suggest that certain tumor necrosis factor alleles are associated with DH (Messer *et al*, 1994). It is possible that specific cytokine signals produced by inflammatory cells or damaged keratinocytes themselves induce the expression of proteinases in DH lesions.

Type VII collagen was studied in DH lesions because both interstitial collagenase and stromelysin-1 can degrade this anchoring fibril component (Seltzer *et al*, 1989; Sawamura *et al*, 1991). Clear disruption of type VII collagen, although not as extensive as that of laminin-1, for example, was evident at 24 h, but not at 12 h. Thus, our results in 24-h DH blisters do not agree with those of Haapalainen *et al* (1995), who reported that in four spontaneous DH blisters, type VII collagen remained intact.

Staining for laminin-5 showed co-localization with collagenase mRNA in basal keratinocytes. Laminin-5, a component of anchoring filaments, is essential for adhesion of keratinocytes to the basal lamina (Rousselle *et al*, 1991) and is produced by migrating epidermal cells at the wound edge (Larjava *et al*, 1993). Laminin-5 co-localizes with receptor for uPA in the invading front of carcinoma cells (Pyke *et al*, 1995). In DH lesions at 24 h, laminin-5 was not found in the basement membrane but was produced by keratinocytes, as demonstrated by cytoplasmic staining of these cells **(Figs 1*G*, 2*A*, 3*A*)**. Production of laminin-5 may thus be a protective response of basal keratinocytes to destruction of underlying basement membrane components. Furthermore, because

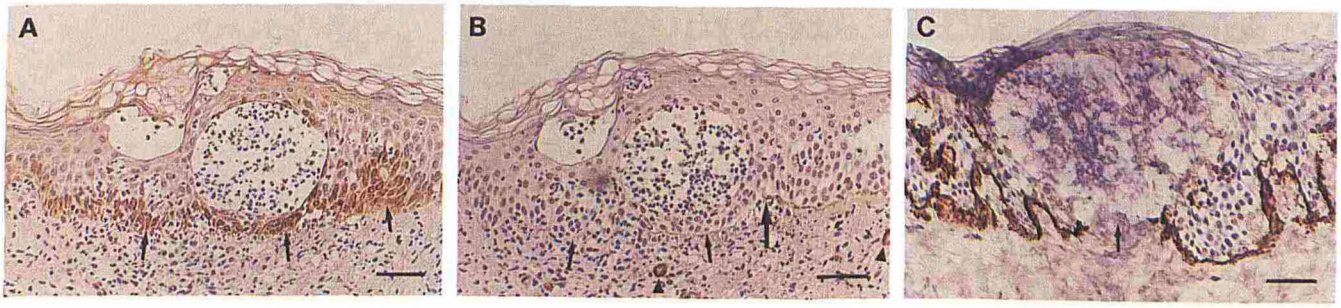

Figure 3. Laminin-5, laminin-1, and type VII collagen are disrupted in lesional areas. Immunohistochemical staining for laminin-5 *(A)*, laminin-1 *(B)*, and type VII collagen *(C)* in a 24-h blister induced with the 50% potassium iodide patch test. *A*, intracellular laminin-5 in cells surrounding a neutrophilic abscess *(arrows)*, while staining in the basement membrane is absent. *B*, disrupted staining for laminin-1 in lesional area *(small arrows)*. *Large arrow* marks the end point of normal laminin-1 staining. Staining around small vessels remains continuous *(arrowheads)*. *C*, disruptions in type VII collagen in the area directly beneath blisters *(arrow)*. *Scale bars*: *A–C*, 19 μm.

laminin-5 has been shown to inhibit keratinocyte motility *in vitro*,[1] its production may indicate cessation of migration of basal keratinocytes in DH blisters.

The results of this study further substantiate the role of uPA in activating metalloenzymes *in vivo* and the role of stromelysin-1 in basement membrane degradation in DH lesions. Furthermore, interstitial collagenase may take part in the degradation of type VII collagen in DH. Laminin-5 is upregulated in basal keratinocytes in the vicinity of developing blisters, suggesting that in addition to dermal wound repair, laminin-5 may participate in epithelial regeneration associated with the healing of superficial blisters.

We thank Dr. Gregory Goldberg for the interstitial collagenase cDNA, Dr. Markku Kurkinen for the stromelysin-1 cDNA, Dr. Henning Birkedal-Hansen for the stromelysin-2 cDNA, Dr. Karl Tryggvason for the laminin-5 antibodies, Dr. Arja-Leena Kariniemi for her pathology expertise, and Ms. Alli Tallqvist for her excellent technical assistance. This work has been supported by Finska Läkaresällskapet, the Sigrid Juselius Foundation, the Paulo Foundation, and the Helsinki University Central Hospital Research Fund.

REFERENCES

Airola K, Vaalamo M, Reunala T, Saarialho-Kere UK: Enhanced expression of interstitial collagenase, stromelysin-1, and urokinase plasminogen activator in lesions of dermatitis herpetiformis. *J Invest Dermatol* 105:184–189, 1995

Apte SS, Olsen BR, Murphy G: The gene structure of tissue inhibitor of metalloproteinases (TIMP)-3 and its inhibitory activities define the distinct TIMP gene family. *J Biol Chem* 270:14313–14318, 1995

Blenkinsopp WK, Haffenden GP, Fry L, Leonard JN: Histology of linear IgA disease, dermatitis herpetiformis, and bullous pemphigoid. *Am J Dermatopathol* 5:547–554, 1983

Brown LF, Harrist TJ, Yeo K-T, Ståhle-Bäckdahl M, Jackman RW, Berse B, Tognazzi K, Dvorak HF, Detmar M: Increased expression of vascular permeability factor (vascular endothelial growth factor) in bullous pemphigoid, dermatitis herpetiformis, and erythema multiforme. *J Invest Dermatol* 104:744–749, 1995

Buttice G, Kurkinen M: A polyomavirus enhancer A-binding protein-3 site and Ets-2 protein have a major role in the 12-O-tetradecanoylphorbol-13-acetate response in the human stromelysin gene. *J Biol Chem* 268:7196–7204, 1993

Graeber M, Baker BS, Garioch JJ, Valdimarsson H, Leonard JN, Fry L: The role of cytokines in the generation of skin lesions in dermatitis herpetiformis. *Br J Dermatol* 129:530–532, 1993

Haapalainen T, Oksala O, Kallioinen M, Oikarinen A, Larjava H, Salo T: Destruction of the epithelial anchoring system in lichen planus. *J Invest Dermatol* 105:100–103, 1995

Haffenden GP, Blekinsopp WG, Ring NP, Wojnarowska FP, Fry L: The potassium iodide patch test in dermatitis herpetiformis in relation to treatment with a gluten-free diet and dapsone. *Br J Dermatol* 102:313–317, 1980

Inoue M, Kratz G, Haegerstrand A, Ståhle-Bäckdahl M: Collagenase expression is rapidly induced in wound-edge keratinocytes after acute injury in human skin, persists during healing, and stops at re-epithelialization. *J Invest Dermatol* 104: 479–483, 1995

Karttunen T, Autio-Harmainen H, Räsänen O, Risteli J, Risteli L: Immunohistochemical localization of epidermal basement membrane laminin and type IV collagen in bullous lesions of dermatitis herpetiformis. *Br J Dermatol* 111:389–394, 1984

Larjava H, Salo T, Haapasalmi K, Kramer RH, Heino J: Expression of integrins and basement membrane components by wound keratinocytes. *J Clin Invest* 92:1425–1435, 1993

Messer G, Kick G, Ranki A, Koskimies S, Reunala T, Meurer M: Polymorphism of the tumor necrosis factor genes in patients with dermatitis herpetiformis. *Dermatology* 189(suppl 1):135–137, 1994

Murphy G, Ward R, Gavrilovic J, Atkinson S: Physiological mechanisms for metalloproteinase activation. *Matrix* (suppl)1:224–230, 1992

Prosser IW, Stenmark KR, Suthar M, Crouch EC, Mecham RP, Parks WC: Regional heterogeneity of elastin and collagen gene expression in intralobar arteries in response to hypoxic pulmonary hypertension as demonstrated by *in situ* hybridization. *Am J Pathol* 135:1073–1088, 1989

Pyke C, Salo S, Ralfkiaer E, Romer J, Dano K, Tryggvason K: Laminin-5 is a marker of invading cancer cells in some human carcinomas and is coexpressed with the receptor for urokinase plasminogen activator in budding cancer cells in colon adenocarcinomas. *Cancer Res* 55:4132–4139, 1995

Reitamo S, Reunala T, Konttinen YT, Saksela O, Salo OP: Inflammatory cells, IgA, C3, fibrin and fibronectin in skin lesions in dermatitis herpetiformis. *Br J Dermatol* 105:167–177, 1981

Rousselle P, Lunstrum GP, Keene DR, Burgeson RE: Kalinin: an epithelium-specific basement membrane adhesion molecule that is a component of anchoring filaments. *J Cell Biol* 114:567–576, 1991

Saarialho-Kere UK, Chang ES, Welgus HG, Parks WC: Distinct localization of collagenase and TIMP expression in wound healing associated with ulcerative pyogenic granuloma. *J Clin Invest* 90:1952–1957, 1992

Saarialho-Kere UK, Chang ES, Welgus HG, Parks WC: Expression of interstitial collagenase, 92 kDa gelatinase, and TIMP-1 in granuloma annulare and necrobiosis lipoidica diabeticorum. *J Invest Dermatol* 100:335–342, 1993a

Saarialho-Kere UK, Kovacs SO, Pentland AP, Olerud JE, Welgus HG, Parks WC: Cell-matrix interactions modulate interstitial collagenase expression by human keratinocytes actively involved in wound healing. *J Clin Invest* 92:2858–2866, 1993b

Saarialho-Kere UK, Pentland AP, Birkedal-Hansen H, Parks WC, Welgus HG: Distinct populations of basal keratinocytes express stromelysin-1 and stromelysin-2 in chronic wounds. *J Clin Invest* 94:79–88, 1994

Saarialho-Kere UK, Vaalamo M, Airola K, Niemi K-M, Oikarinen AI, Parks WC: Interstitial collagenase is expressed by keratinocytes which are actively involved in re-epithelization in blistering skin diseases. *J Invest Dermatol* 104:982–988, 1995

Salo OP, Laiho K, Blomqvist K, Mustakallio KK: Papillary deposition of fibrin in iodide reactions in dermatitis herpetiformis. *Ann Clin Res* 2:19–21, 1970

Sawamura D, Sugawara T, Hashimoto I, Bruckner-Tuderman L, Fujimoto D, Okada Y, Utsumi N, Shikata H: Increased gene expression of matrix metalloproteinase-3 (stromelysin) in skin fibroblasts from patients with severe recessive dystrophic epidermolysis bullosa. *Biochem Biophys Res Commun* 174:1003–1008, 1991

Seltzer JL, Eisen AZ, Bauer EA, Morris NP, Glanville RW, Burgeson RE: Cleavage of type VII collagen by interstitial collagenase and type IV collagenase (gelatinase) derived from human skin. *J Biol Chem* 264:3822–3826, 1989

Silbiger SM, Jacobsen VL, Cupples RL, Koski RA: Cloning of cDNA encoding human TIMP-3, a novel member of the tissue inhibitor of metalloproteinase family. *Gene* 141:293–297, 1994

Ståhle-Bäckdahl M, Inoue M, Giudice GJ, Parks WC: 92-kD gelatinase is produced by eosinophils at the site of blister formation in bullous pemphigoid and cleaves the extracellular domain of recombinant 180-kD bullous pemphigoid autoantigen. *J Clin Invest* 93:2022–2030, 1994

Ståhle-Bäckdahl M, Parks WC: 92 kDa gelatinase is actively expressed by eosinophils and secreted by neutrophils in invasive squamous cell carcinoma. *Am J Pathol* 142:1–6, 1993

Vassalli J-D, Sappino A-P, Belin D: The plasminogen activator/plasmin system. *J Clin Invest* 88:1067–1072, 1991

[1] O'Toole EA, Marinkovich MP, Shervin N, Woodley DT: Laminin-5 inhibits keratinocyte motility. *J Invest Dermatol* 106:822, 1996 (abstr).